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EFFECTS OF CYANIDE AND 2 DEOXYGLUCOSE ON PROXIMAL TUBULAR FUNCTION IN THE RAT KIDNEY

By

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June 1969

U. S. ARMY AEROMEDICAL RESEARCH LABORATORY
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A series of 13 experiments were performed to study the effects of cyanide, an oxidative inhibitor, and 2 deoxyglucose (2DG), a glycolytic inhibitor, on the function of the proximal tubule of the rat's kidney. The technique utilized was split oil droplet microperfusion of surface proximal nephron segments with sequential photomicrography. Isotonic saline was the control perfusion fluid. Cyanide reduced the reabsorptive rate of the perfused nephron segments to 50% of the control. 2DG had no effect on saline reabsorption. Cyanide plus 2DG perfused simultaneously in saline inhibited reabsorption to the same degree as did cyanide alone.

These results are interpreted as indicating almost total dependence of proximal tubular reabsorption of filtrate upon energy available from oxidative metabolism. Since reabsorption of filtrate in this segment is mediated through active sodium transport, it would appear that oxidative metabolism and not glycolysis is the energy source for this process.

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EFFECTS OF CYANIDE AND 2 DEOXYGLUCOSE ON PROXIMAL TUBULAR FUNCTION IN THE RAT KIDNEY

INTRODUCTION

Evidence suggests that the mammalian kidney is dependent upon oxidative metabolism as well as glycolysis for the energy utilized in transporting sodium ion (1) (2) (3). Recent work performed by this investigator provides evidence of a difference in the contribution of these two sources of energy in the different segments of the nephron (4).

As a means of confirming and extending the conclusions reached from the previous studies the experiments included in this report were performed.

Since the proximal tubule is the one primarily involved in acute tubular necrosis, it is probable that these results will provide information as to the causes of the lesions seen in this disease entity.

METHODS

White Charles River rats weighing 170-310 gms were anaesthetized with Inactin 80 mg per Kgm body weight. Cannullae were inserted into one jugular vein and the trachea. The left kidney was exposed through an abdominal-flank incision and mounted in a plastic cup. The surface of the kidney was continuously bathed in mineral oil preheated to maintain kidney temperature at 37°C. The experiment was conducted on a water heated animal board maintained at 37°C. Fluid replacement during surgery and thereafter consisted of intravenous isotonic saline amounting to 1-1.5% of the rat's body weight.

The technique for measuring proximal tubular reabsorptive function was the split oil droplet microperfusion technique reported by Gertz (14). Double lumened micropipettes were fabricated as described by Windhager (15). One side of the pipette was filled with Sudan Black stained castor oil, the other with the test perfusion fluid. This was manipulated under microscopic vision into a surface proximal tubular segment. The segment was filled with castor oil and the oil split with a length of perfusion fluid. The rate of reabsorption of this perfusion droplet was recorded by sequential photomicrography utilizing a 35mm motorized camera adapted to the microscope. Exposure intervals were controlled via an interval timer. Lighting was via an electronic flash unit synchronized with the camera shutter. High Speed Ektachrome, Type B, was used. The resulting slides

were utilized to measure the fluid droplet size at each interval. This was plotted on semilog graph paper as a function of time, and a reabsorptive half time (T 1/2) determined from the graph of each perfusion sequence.

RESULTS

In this series of 13 experiments isotonic saline was the basic control perfusion fluid. The reabsorptive mean T 1/2 for this and the other solutions tested are included in Table I. The mean half time for isotonic saline and for isotonic saline plus glucose were not statistically different, their values being 9.2 + 0.4 seconds and 9.6 + 0.5 seconds respectively. Addition of 2DG to the isotonic saline produced no change from the control reabsorptive rate, T 1/2 = 9.2 + 0.7 seconds. Addition of 10^{-4}M potassium cyanide approximately doubled the half time yielding a mean of 20.3 + 1.4 seconds. Addition of cyanide plus 2DG had no greater effect than for cyanide alone, with a T 1/2 of 18.4 + 0.9 SEM.

DISCUSSION

In these experiments the effects of specific metabolic inhibitors on the reabsorption of isotonic saline by the proximal tubule were studied. The resulting data provides evidence concerning the metabolic basis for active sodium transport in this nephron segment since it has been well established that filtrate reabsorption is dependent upon active sodium transport in the proximal convolution of the mammalian kidney (5) (6) (7) (8).

Previous experiments utilizing kidney slice preparations (1) (2), renal clearance techniques (9) (10) (11), and micropuncture techniques (7) (12) provide evidence that oxidative metabolism is required for proximal tubular sodium reabsorption. Previous work performed by this investigator suggests that glycolytic metabolism makes little or no contribution to sodium transport in this segment (4). However, there is no evidence to date ruling out glycolysis as a partial source of metabolic energy for this process and no evidence concerning the possibility of a cellular shift to glycolysis upon inhibition of oxidative metabolic sequences.

The inhibitory effects of cyanide alone confirm the observations made by Chertock et al (12) and reinforce those previously reported by this investigator (4). This suggests that oxidative metabolism is essential for active sodium transport in the proximal tubule of the rat, since KCN inhibits the electron transport system in the dose used in these perfusions (16). The 2DG, a specific inhibitor of glycolysis (13), had no measurable effect on proximal tubular sodium transport.

The combined effect of this inhibitor with cyanide was no greater than for cyanide alone. This is interpreted to provide evidence that glycolysis makes no obligatory contribution to proximal sodium reabsorption and that no measurable shift to glycolysis occurs when oxidative metabolism is reduced.

These results suggest the possibility that proximal tubular metabolism and transport are linked in a specific fashion. It still remains unclear whether this linkage is based upon the specific requirements of sodium transport in this segment of the nephron or is merely a result of a limitation imposed by the quantity of energy available from glycolytic vs. oxidative metabolism. In order to decide between these two, further experiments are required.

The clinical implications of these studies are significant. The proximal tubule of the mammalian kidney appears obligatorily dependent upon oxidative metabolism for functional integrity. A limitation in the capacity of this segment to survive might evolve from an anoxic environment or from conditions depriving the cell of oxidative metabolic sequences. This correlates well with the observation that the proximal tubule is the primary site of damage in acute tubular necrosis. However, the tentative nature of such a carryover of data from animal research to human conditions requires further studies both at the clinical level and with the rat kidney. These might be directed toward the nature of the linkage of sodium transport to metabolic energy production and toward applicability of these results to function in the human kidney.

TABLE I

Perfusion Fluid	T 1/2 ± SEM ( ) Seconds	"t" Test
Isotonic NaCl	9.2 ± 0.4 (31)	
Isotonic NaCl + 10mM Glucose	9.6 ± 0.7 (19)	vs Isot. NaCl P = 0.50
Isotonic NaCl + 10mM 2DG	9.2 ± 0.7 (19)	vs. Isot. NaCl + Gluc. 0.60 P 0.70
Isot. NaCl + 10^{-4}M KCN + 10mM Gluc	20.3 ± 1.4 (20)	vs. Isot. NaCl + Gluc. P 0.001
Isot. NaCl + 10^{-4}M KCN + 10mM 2DG	18.4 ± 0.9 (18)	vs. Isot. NaCl + KCN + Gluc 0.40 P 0.50

LEGEND

Effects of KCN, 2 deoxyglucose on reabsorption of isotonic saline perfusate proximal tubular segments.

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